PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : C12N 5/00, A61K 31/735	A1	(11) International Publication Number: WO 90/00596 (43) International Publication Date: 25 January 1990 (25.01.90)

(21) International Application Number: PCT/US89/02944

(22) International Filing Date: 6 July 1989 (06.07.89)

(30) Priority data:
216,176 7 July 1988 (07.07.88) US
Not furnished 28 June 1989 (28.06.89) US

(71) Applicant: THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US/US]; 133 South 36th Street, Philadelphia, PA 19104 (US).

(72) Inventors: ROTH, Steven, A. ; 1105 Rose Glen Road, Gladwyne, PA 19035 (US). WEISZ, Paul, B. ; 3 Delaware Rim Drive, Yardley, PA 19067 (US). FARNBACH, George ; 132 Rutgers Avenue, Swarthmore, PA 19081 (US). WEINER, David, B. ; 23 Henley Road, Penn Wynn Hills, PA 19401 (US). GREENE, Mark, I. ; 300 Righters Mill Road, Penn Valley, PA (US). WILLIAMS, William, V. ; 2408 Avon Road, Ardmore, PA 19003 (US).

(74) Agent: MISROCK, S., Leslie; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), BF (OAPI patent), BJ (OAPI patent), CF (OAPI patent), CG (OAPI patent), CH (European patent), CM (OAPI patent), DE (European patent), DK, FR (European patent), GA (OAPI patent), GB (European patent), HU, IT (European patent), JP, KR, LU (European patent), ML (OAPI patent), MR (OAPI patent), NL (European patent), NO, SE (European patent), SN (OAPI patent), TD (OAPI patent), TG (OAPI patent).

Published
With international search report.

(54) Title: METHOD OF MODULATING VIRUS-HOST CELL INTERACTIONS USING CARBOHYDRATES AND CARBOHYDRATE DERIVATIVES

(57) Abstract

The invention discloses novel methods of inhibiting infection of cells by a virus. Carbohydrate blocking agents capable of interacting with the cells or virus are provided which are contacted with the cells under conditions selected to effect interaction of the carbohydrate blocking agent with the cells or virus resulting in interference with binding of the virus with the cells. Suitable carbohydrate blocking agents include but are not limited to linear and cyclodextrins as well as chemically modified derivatives thereof. The invention also provides methods of inhibiting formation of syncytia between cells or virus utilizing linear and cyclodextrins and chemically modified derivatives thereof. The invention further provides methods of inhibiting viral transcriptase utilizing chemically modified derivatives of cyclodextrins. The invention also discloses compositions comprising alpha-, beta-, or gamma-cyclodextrin in combination with nucleoside derivatives or a steroid or non-steroid growth modulating agent which may be used to inhibit virus infection and syncytia formation.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Fasso
BG	Bulgaria
BJ	Benin
BR	Brazil
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CM	Cameroon
DE	Germany, Federal Republic of
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
HU	Hungary
IT	Italy
JP	Japan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
MC	Monaco
MG	Madagascar
ML	Mali
MR	Mauritania
MW	Malawi
NL	Netherlands
NO	Norway
RO	Romania
SD	Sudan
SE	Sweden
SN	Senegal
SU	Soviet Union
TD	Chad
TG	Togo
US	United States of America

METHOD OF MODULATING VIRUS-HOST CELL INTERACTIONS USING CARBOHYDRATES AND CARBOHYDRATE DERIVATIVES

1. FIELD OF THE INVENTION

The present invention relates to the field of treatments for infection by viruses. More particularly, this invention relates to the field of biologically active agents which interfere with or inhibit virus-host cell interactions by binding to receptor site(s) on the host cells or on viruses or both.

2. BACKGROUND OF THE INVENTION

2.1. HIV

Acquired Immune Deficiency Syndrome (AIDS) is one of the most feared diseases in the world today. Infection with the human immunodeficiency virus (HIV-1), believed to be the cause of AIDS is almost always fatal. Symptoms of the disease can take years to develop, thus facilitating the spread of this fatal disease by persons unknowingly harboring the virus. Treatments for AIDS are limited and have been unsuccessful in controlling the disease.

It is generally recognized that an envelope glycoprotein of HIV-1 plays a key role in the process of infection of human tissue cells by the virus. The envelope glycoprotein consists of two main glycoprotein portions-gp120 and gp41. The gp120 is believed to be the outermost part of the complex made up of these two glycoproteins. It is believed that the binding of the HIV-1 particle to human cells, a key event in the infection of cells with the virus, requires binding between gp120 and a receptor protein complex known as CD4 on certain human cells.

CD4 expression on human cells has been demonstrated to increase susceptibility to HIV-1 binding, penetration and infection. CD4, a 55,000 dalton cell surface glycoprotein is thought to represent a cell surface receptor to which HIV-1

envelope glycoprotein (gp120) binds. This interaction likely facilitates events necessary for viral fusion with the plasma membrane and subsequent entry into the cell.

It is believed that there are at least three separate binding sites on the gp120 molecule which combine to form a high affinity binding site for the CD4 receptor. This affinity for the CD4 receptor is extremely strong and this binding affinity may displace or prevent binding of antibodies produces by the host in standard immunological defense. For example, most AIDS patients and most ARC (AIDS Related Complex) patients have high levels of antibody to gp120, however the gp120-CD4 interaction may displace bound antibody so that vaccines directed against gp120 can be expected to be ineffective since the antibodies they elicit from the host will be relatively ineffective. One commercially available compound which is useful as an anti-infective agent is a form of CD4. The compound is believed to work because it binds the gp120 as tightly as the natural CD4. In this way, if given in high enough concentrations, the free (administered) CD4 will bind all of the viral gp120 and prevent its binding CD4 on host cells.

The HIV-1 virus has been shown to infect preferentially cells expressing the CD4 surface glycoprotein. This tropism is believed to result from interactions between the virus envelope gp120 and a CD4 high affinity binding site glycoprotein which permits viral absorption. Following the initial attachment of virus to the cell surface CD4 molecule, other regions of the virus envelope are believed to be important in initiating fusion between the viral and cellular membranes. This interaction is thought to precede viral entry, uncoating, and replication.

Although there is much evidence to support the foregoing proposed sequence of events, several studies have demonstrated that CD4 may be necessary, but not sufficient, to mediate entry of HIV-1 into cells. These experiments have

used primary cells expressing CD4 and human cell lines transfected with CD4; these cells bind and internalize HIV-1 virus. Additionally, monocytes and certain rare B cell lines which are infectable by HIV-1 express mRNA potentially encoding CD4 proteins. Finally, antibodies to specific CD4 epitopes inhibit viral attachment and syncytia formation as well as viral infection.

Most murine cells expressing the human CD4 glycoprotein bind HIV-1 but do not readily form syncytia and are not readily infected. Additionally, it has been reported that antibodies that disrupt viral-CD4 interactions do not block viral infection of certain human neuronal cell lines. These latter observations suggest that other factors and or other molecules present on human cells in addition to CD4 may be operative in HIV-1 penetration.

Cell surface receptors are known to occur on many types of cells. These receptors are believed to be important in a wide variety of cellular functions such as cell-cell recognition processes, growth regulation, metabolic and hormonal regulation and structural aggregation of cells. Some of these cell surface receptors, such as the CD4 receptor, are considered to be glycoproteins. Glycoproteins are molecules comprising a protein moiety linked with a polysaccharide moiety. Typically, the protein moiety constitutes the greater part of the molecule. The ligands of some receptors, such as gp120, the envelope glycoprotein of HIV-1 which binds to the receptor CD4, are also glycoproteins. It is believed that the polysaccharide portion of the glycoprotein molecule plays a role in the interaction between the receptor and its ligand and is important for imparting specificity to the receptor-ligand binding in at least some instances.

2.2. POLYSACCHARIDES AS THERAPEUTIC AGENTS

Polysaccharides are biologically important polymers made up of linked single sugar molecules. These substances are ubiquitous in biological organisms. Starch is a commonly known polysaccharide as are many naturally-occurring compositions. Small segments of polysaccharide chains are known to be important in the physical and chemical properties of many proteins-particularly in eukaryotic organisms. Various polysaccharides and their derivatives are known to be important in surface phenomena in biological systems. Heparin, for example is a mixture of polysulfated polysaccharides with molecular weights ranging from about 5,000 to 40,000; it is commercially prepared from biological material such as lung tissue. The substances contained in heparin are important biological surfactants and also prevent clot formation in blood. Heparin and heparin-like substances are also known to prevent the adhesion of potentially infectious bacteria to surfaces in the urinary bladder, urethra, and ureters.

Although heparin has long been used in medicine to treat blood clots, heparin has recently received attention by researchers for other uses. Science Focus, New York Academy of Sciences, 2(3): 1, 1988 reports that heparin can inhibit HIV, the viral agent that causes AIDS, in selected cells in one stage of the virus's reproductive cycle. However, heparin is a well-known, strong anticoagulant and its continued use in mammals can lead to bleeding disorders. Further, commercial heparin is a heterogeneous mixture of substances derived from natural sources.

Mitsuya et al., Science, 240: 646-649, (1988), have demonstrated that the use of high molecular weight (approximately 8,000 M.W.) poly-sulfated, linear poly-dextrans can inhibit the infection by HIV-1 of certain human white blood cells. Baba et al., Proc. Natl. Acad. Sci. U.S.A. 85. 6132-6136 have demonstrated that dextran sulfate

(5000 m.w.) and heparin can inhibit the infection by HIV-1 of certain white blood cells. U.S. Patent No. 4,783,446, 1988, discloses the use of carrageenans or mixtures of carrageenans to treat AIDS and other retrovirus infections. The carrageenans will be in the range of 5,000-500,000 M.W. with most ranging from 100,000 to 500,000 M.W. However, it is not clear whether this phenomenon is a specific or non-specific effect of this compound, since such large molecular weight substances of this type may have non-specific membrane effects on the lipid properties of the cell surface and the viral envelope surface. Further, like heparin, the high molecular weight compound is strongly anticoagulant.

Treatment of individuals infected with HIV-1 has been complicated by the binding capacity of the virus to mammalian cells and the extreme toxicity of infection with the virus. There is a potential for inadvertently infecting healthy individuals with only partially inactivated whole HIV-1 or components of the virus as part of a vaccine. Efforts to control the virus through drugs has not succeeded to date. Alternative means of treating individuals infected with HIV-1, as well as alternate means of preventing or inhibiting infection of cells with HIV-1 are needed which are not toxic to the individual infected with HIV-1 and are safe for individuals not infected with the virus.

3. SUMMARY OF THE INVENTION

The present invention provides novel methods for inhibiting infection of cells by a virus using a carbohydrate as a blocking agent. The invention is also directed to methods for inhibiting the cell to cell transmission of the virus using a carbohydrate as a blocking agent. The carbohydrate blocking agent is capable of interacting with the host cells and interferes with binding of the virus to the cells. Preferably, the carbohydrate blocking agent is a very water soluble derivative of a cyclic oligosaccharide

having up to about twelve sugar monomers. The cyclic oligosaccharide derivative may contain ionic and/or non-ionic substitutes. As used in the present application the term "very water soluble" is intended to encompass a solubility, measured at 0°C, of at least about 15 gm/100 ml in distilled water, preferably at least about 20 gm/100 ml, more preferably greater than 30 gm/100 ml. Preferably, the cyclic oligosaccharide has about six to eight sugar monomers.

This invention also provides a method of inhibiting syncytia formation between cells comprising providing a carbohydrate blocking agent which is capable of interacting with said cells or virus, said carbohydrate blocking agent comprising a cyclic oligosaccharide derivative having up to about twelve sugar wherein said derivative is characterized by a solubility of at least 20 gm/100 ml. The agent is administered to said cells under conditions selected to effect interaction of the cyclic oligosaccharide derivative with said cells thereby inhibiting formation of syncytia. In another embodiment, the cyclic oligosaccharide derivatives of the present invention may inhibit syncytia formation by administering the carbohydrate blocking agent under conditions where the virus is blocked from binding to the cell.

This invention further provides a composition for inhibiting virus infection in mammals including humans, comprising (1) a very water-soluble derivative of a cyclic oligosaccharide in combination with (2) at least one antiviral agent, said derivative being characterized by a solubility in distilled water of at least about 20 grams per 100 milliliters of water at 0°C. The compositions may also be used to inhibit cell to cell transmission of the virus. The compositions in another embodiment may be used to inhibit syncytia formation in infected cells.

The invention additionally provides a composition for inhibiting retrovirus infection in mammals including humans comprising (1) a very water-soluble derivative of a cyclic oligosaccharide in combination with (2) at least one cell growth modulating steroid or non-steroidal organic compound, wherein the steroid compound is devoid of glucocorticoid activity, and said derivative is characterized by a solubility in distilled water of at least 20 grams per 100 milliliters of water at 0°C. In one embodiment, these compositions may be used to treat Kaposi's sarcoma, often manifested in AIDS patients. The compositions may also be used to prevent cell to cell transmission of the virus.

Further provided is a composition for inhibiting retrovirus infection in mammals including humans, comprising (1) a very water-sluble derivative of a cyclic oligosaccharide in combination with (2) at least one antiviral agent and (3) at least one growth modulating steroid or non-steroidal organic compound, wherein the steroid compound is devoid of glucocorticoid activity, and said derivative is characterized by a solubility in distilled water of at least 20 grams per 100 milliliters of water of 0°C. In one embodiment, these compositions may be used to treat Kaposi's sarcoma, often manifested in AIDS patients.

4. BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows syncytia formation in the presence (A) or absence (B) of 320 μM β-cyclodextrin sulfate.

Figure 2 illustrates the effects of cyclodextrin and cyclodextrin derivatives on HIV infectivity as determined by reverse transcriptase activity. Samples assayed are (A) untreated control cells, (B) β-cyclodextrin unsubstituted ring, (C) partially sulfated β-cyclodextrin ring, (D) propylated β-cyclodextrin ring, (E) sulfated β-cyclodextrin ring, and (F) methylated β-cyclodextrin ring. Numbers indicate 1/2 dilutions of test compounds with 1 = 2.5 mM concentration of compound.

Figure 3 shows the effect of beta-cyclodextrin tetradecasulfate (β-CD-14S or β-CD-TDS) on HIV RNA production. Virus particles were incubated with target cells in the presence or absence of 1M of β-CD-14S. At 72 hours post infection, the cells were washed and dot blot assays were performed to determine the presence or absence of viral RNA production. In (A), target cells were infected in the presence of sulfated β-cyclodextrin (lane A), in the absence of cyclodextrins (lane B), or in the presence of unsubstituted β-cyclodextrin (lane C). 1/2 dilutions of cells are from left to right starting with 10^6 cell/well utilized. In (B), virally infected cells (+) or uninfected cells (-) were assayed for their ability to produce viral RNA by dot blot analysis. CD = infected cells incubated with 1 mM of β-CD-14S. 1/2 dilutions of cells are from top to bottom starting with 10^6 cell/well.

Figure 4 shows an electron micrograph of HIV-1 particles produced by infected H9 cells in the presence of 1 mM of β-CD-14S. Note the mature morphology of the viral particles produced (upper left) as well as budding virus.

Figure 5 shows monoclonal antibody binding in the presence of 1 μM of β-CD-14S. The human T cell line CEM was preincubated with 1 M of β-CD-14S, and then incubated with various monoclonal antibodies as noted in Section 6.1, infra. Fluorescence histograms show relative fluorescence intensity (abscissa) versus relative cell number (ordinate) for each monoclonal as listed. Primary antibody binding was assessed in the presence (right) or absence (left) of β-CD-14S.

Figure 6 compares the effects of dextran polysulfate and substituted β-cyclodextrins on HIV infectivity. In (A) degree of syncytia inhibition is shown on the ordinate for each compound added. SUL = 600 μM β-CD-14S, AL = 600 μM unsubstituted β-CD, Pro = 600 μM propoxylated β-CD, 2MET = 300 μM methoxylated β-CD, DS5 = 600 μM dextran sulfate 5,000, DS500 = 600 μM dextran sulfate 500,000. Results for each of

3 time points are shown. In (B) similar results utilizing reverse transcriptase assays are shown. Infectious virus particles were incubated in the presence of sulfated β-cyclodextrin (lane 1), dextran sulfate 5000 (lane 2) or media (lane 3) for 6 days at which time cells were resuspended in fresh media and allowed to incubate a further 10 days before being harvested. Reverse transcriptase activity was assayed as described in Section 6.1, infra. 1/2 dilutions of the compound added are shown from bottom (highest concentration at 2.5 mM) to top (lowest concentration).

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods for inhibiting infection of cells by a virus which employ a carbohydrate blocking agent capable of interacting with cells to interfere with the binding of the virus with the cells. The invention also relates to methods for using such carbohydrate blocking agents to block cell to cell transmission of the virus. The invention further provides methods employing such carbohydrate blocking agents to inhibit syncytia formation. The invention further relates to a composition comprising a carbohydrate blocking agent in combination with at least one antiviral agent. Also provided is a composition comprising a carbohydrate blocking agent in combination with at least one steroid or nonsteroidal organic compound where the steroid does not contain glucocorticoid activity.

5.1. CARBOHYDRATE BLOCKING AGENTS: OLIGOSACCHARIDES

The carbohydrate blocking agents suitable for use according to the method of the invention comprise one or more very water soluble derivatives of cyclic oligosaccharide having up to twelve sugar monomers. Oligosaccharides are generally defined as carbohydrates containing two up to about ten simple sugars linked together; polysaccharides as having

more than ten simple sugars linked together. However, for the purposes herein, the term oligosaccharide is used to mean carbohydrates containing from two to about twelve simple sugars linked together. It is preferred that oligosaccharides having less than about twelve sugar monomers be employed. More preferred cyclic oligosaccharides comprise cyclic molecules having six to eight sugar monomers such as alpha-, beta-, or gamma-cyclodextrin. Cyclodextrins (CDs) are cyclic, non-linear oligosaccharides having at least six glucopyranose units. Although cyclodextrins with up to twelve such units are known, only the first three homologs have been studied extensively. The common designations of the lower molecular weight cyclodextrins are alpha-, beta-, and gamma-cyclodextrin which correspond to 6,7 and 8 glucopyranose units, respectively. Cyclodextrins are also known sometimes as cycloamyloses. A number of cyclodextrin derivatives are known. In general, these chemically modified cyclodextrins are formed by reaction of the primary or secondary hydroxyl groups attached to carbons 2, 3, or 6, without disturbing the alpha (1 - 4) hemiacetal linkages. Sugars other than glucose, such as arabinose, galactose, and other 5- and 6-carbon sugars and many other forms known to those of ordinary skill in the art as "sugars" may be useful.

Highly water-soluble cyclic oligosaccharide derivatives bearing non-ionic and/or ionic substituents are useful according to the present invention. Suitable highly water-soluble cyclic oligosaccharide derivatives include alpha, beta, and gamma CD derivatives having non-ionic substituents including but not limited to alkyl substituents such as methyl, ethyl, etc., as well as those in which a number of hydroxyl groups are replaced by other groups so as to increase the hydrophilic activity of CD. Such groups may include esters, ethers (e.g. alkoxy), thioesters, thioethers, carboxylic acids, carbohydrates, or other groups which convey hydrophilic activity by way of polar or hydrogen bonding

constituents. Substitution may occur at some or all of the hydroxyl groups. In a preferred embodiment, the cyclic oligosaccharides contain about 10 to about 16 groups.

The cyclic oligosaccharide derivatives useful in the present invention are highly hydrophilic and therefore very water soluble. A highly hydrophilic character probably is important to allow interaction with cellular surfaces. The hydrophilic activity is likely roughly indicated by the affinity to water, as measured by water solubility. It is important to measure the same at 0°C since at higher temperatures the most suitable derivative have solubilities so high that meaningful measurements are difficult.

It is contemplated that very water-soluble cyclic oligosaccharide derivatives bearing ionic and/or non-ionic substituents may in some instances have advantageous properties, and that these are within the scope of this invention. Although highly water-soluble derivatives in general are believed useful, salt derivatives are preferred. The term "very soluble" as used herein refers to a solubility of at least 20 gm/100 ml measured at 0°C, preferably more than 30 gm/100 ml.

The phrase "salt derivative" as used herein means an ionic compound derived from a cyclic oligosaccharide by reaction with a suitable reagent. The preferred salt derivatives are provided by a cyclic oligosaccharide having substituents selected from the group consisting of sulfate, phosphate, carboxylate and mixtures thereof associated with a nontoxic, physiologically acceptable cation. Many of said preferred derivatives are known compounds. (Croft and Bartech, 1983, Tetrahedron 39:1417-1474). But many potentially useful forms may be variants, structurally or chemically of known compounds. Some of the preferred salt forms of the derivatives are the sodium and potassium forms, since these tend to impart increased water solubility to organic anions. The salt derivatives useful herein will

exhibit electrolytic conductivity and osmotic properties characteristic of electrolytes and polyelectrolytes when in aqueous solution. A particularly preferred salt derivative would be a beta cyclodextrin derivative containing about 12-16 sulfate groups. Various degrees of sulfation per glucose unit can be employed, such as an average of one sulfate group per two glucose units or of two sulfate groups per glucose unit. Cyclodextrins having about two sulfate groups per glucose unit are preferred.

The foregoing has been set forth in order to provide a general understanding of the oligosaccharides which are likely useful for the practice of the present invention. It should be understood, however, that such oligosaccharides are described best by what they do rather than by what they are. The oligosaccharides which are useful herein are those oligosaccharides which are capable of interacting with potential host cells in a fashion which interferes with binding of viruses to the host cells. Those skilled in the art will recognize that the manner of interference with binding of a virus to a host cell will vary according to the particular virus-host cell binding which is involved. Different portions of the oligosaccharides are expected to be involved in binding to potential host cells according to the specific requirements of a particular virus-host cell interaction.

5.2. ANTIVIRAL AGENTS

According to one embodiment of the invention, a very water soluble cyclic oligosaccharide derivative in combination with at least one antiviral agent is employed to interfere with virus interaction with a cell. An antiviral agent is any organic or inorganic compound that inhibits any stage of the viral replication cycle including but not limited to the binding of the virus to the cell, entry of the virus into the cell, uncoating of the virus, inhibition of

the replication of the viral nucleic acid, inhibition of viral protein synthesis, and maturation of the virus particle.

An example of an antiviral agent is a nucleoside derivative. Nucleoside derivatives are modified forms of the purine (adenosine and guanosine) and pyrimidine (thymidine, uridine, and cytidine) nucleosides which are the building blocks of RNA and DNA. The nucleoside derivatives which can be utilized in the currently claimed invention include but, are not limited to: AZT (3'-azido-2',3'-dideoxythymidine, azidothymidine, zidovudine);

2',3' dideoxynucleosides including 2',3' dideoxyadenosine, 2',3' dideoxyguanosine, 2',3' dideoxycytidine, 2',3' dideoxythymidine, and 2',3' dideoxyinosine;

3'-deoxythymidine-alene (3'-deoxy-2',3'-didehydrothymidine; and

D4T (2',3'-didehydro-2',3'-dideoxythymidine).

Other examples of nucleoside derivatives include but are not limited to a CD4 peptide or analog thereof and hypericin.

5.3. STEROIDS AND NONSTEROIDAL ORGANIC COMPOUNDS

The present invention provides for any composition which comprises a cyclic oligosaccharide derivative alone or in combination with a steroid or non-steroid organic compounds that are cell growth modulators, i.e. a compound that is capable of altering or changing growth behavior, including both promotion and inhibition.

Most preferred among the steroid growth modulating compounds are those latent growth-inhibiting steroids which lack glucocorticoid and mineralo-corticoid activity, since such activity is an undesired side effect and limits the dose size or extent of use of the steroid for the purpose of the present invention. Among such more preferred steroids are 11

alpha,17,21-trihydroxypregn-4-ene-3,20-dione (11-desoxycortisol or cortexolone), 17 alpha,21-dihydroxypregna-4,9(11)-diene-3,20-dione and tetrahydracortisol.

Non-steroid organic compounds may include, but are not limited to compounds which may be peptide, carbohydrate, or lipid in nature. Examples of such compounds may include but are not limited to interleukin-1, interleukin-2, and interferons alpha, beta, and gamma.

5.4. APPLICATIONS AND METHODS OF USE

The present invention provides novel methods of inhibiting infection of cells by a virus. A carbohydrate blocking agent capable of interacting with host cells is provided to interfere with binding of the virus with the cells. The carbohydrate blocking agent comprises one or more oligosaccharides having up to about twelve sugar monomers. The host cells are contacted with the carbohydrate blocking agent in an amount, for a time and under conditions selected to effect interaction of the carbohydrate blocking agent with the cells to result in the interference with binding of the virus with the cells.

The carbohydrate blocking agent is administered to the cells at a concentration effective to interfere with binding of the virus to the cells. The concentration of any particular oligosaccharide agent administered to cells will depend on such parameters as its solubility in the medium surrounding the cells, its ability to interfere with binding of a virus to the potential host cells and its effects on other biological systems of the cell. It is expected that concentrations ranging from approximately 0.15 to 2 millimolar will result in interference with binding of the virus to the cells. The carbohydrate blocking agents is administered to the cells for a time sufficient to allow and maintain interference of binding of the virus to the cells. Those skilled in the art will recognize that the length of

time that a particular carbohydrate blocking agent will be administered is dependent on such factors as concentration of carbohydrate blocking agent administered, the type of virus-host cell interaction and the ability of the carbohydrate blocking agent to interfere with binding of the virus to the cells.

Conditions under which a particular carbohydrate blocking agent will effect interference with binding of the virus to potential host cells may be determined with suitable assays such as the syncytia inhibition assay described is Section 6.11, _infra_. Such assays will help in determining the concentrations of the carbohydrate blocking agent which are most effective in inhibiting infection of cells with the virus.

Those skilled in the art will recognize that concentrations of the carbohydrate blocking agents may be presented to cells within the body of a mammal, including humans, by any of several routes of administration using dosages which provide at least the above mentioned concentrations in the extracellular medium of the body at the cell surfaces. Such routes of administration include, _inter alia_, oral, intravenous, and transdermal routes.

The methods of the invention are suitable for use to treat infection by viruses. They may be used with retroviruses, including but not limited to human immunodeficiency virus-1 (HIV-1), human immunodeficiency virus-2 (HIV-2), human T-cell lymphotropic virus-I (HTLV-I), human T cell lymphotropic virus (HTLV-II), avian leukosis virus, Rous sarcoma virus, avian erythroblastosis virus, avian myeloblastosis virus, feline leukemia virus, and bovine leukemia virus. They may also be used with paramyxoviruses (e.g. measles virus and respiratory syncytial virus) and herpesviruses (e.g. Epstein Barr virus). Such compounds may

act to interfere with binding of the virus with the cells. Such cells may include but are not limited to lymphoblastoid cells (T cells and B cells) and monocytes.

The inhibition of binding of a virus to a potential host cell is believed to be important in preventing infection of the cell with the virus. In the case of a retrovirus such as HIV-1 or HIV-2, attachment and entry into a host cell are important parts of the life cycle of the virus. If the retrovirus is prevented from entering a cell, the cell will not become infected with the virus. One of the consequences of infection with a virus such as a retrovirus and more particularly HIV-1, is that the cells begin to fuse together forming syncytia. The formation of syncytia is lethal to the affected cells.

It is further believed that some cell surface receptors are also enzymes which recognize certain extracellular substances having short polysaccharide residues. Some of these cell surface receptors are believed to be glycosyltransferases which recognize short polysaccharide residues present in glycoproteins and other biological molecules. It is believed that glycoproteins found on viral surfaces and through which viruses manifest specific cell type or species specific tropisms also use such cell surface receptors/enzymes to bind the host cells prior to infection of the cells.

Prevention of interaction between the virus and potential host cell is important in minimizing the pathophysiology of disease where syncytia formation is an important part of the action of the virus or viral agent in the disease process.

The invention also provides methods for inhibiting cell to cell transmission of a virus. A carbohydrate blocking agent capable of interacting with host cells is provided to interfere with the transmission of virus from one cell to another cell. Examples of such cells include but are not

limited to T cells; B cells, and monocytes. The host cells are contacted with the carbohydrate blocking agent in an amount, for a time and under conditions selected to effect interaction of the carbohydrate blocking agent with the cells to result in the interference with the transmission of the virus from cell to cell. The carbohydrate blocking agent is a very water soluble cyclic oligosaccharide derivative. These methods are suitable for use with retroviruses including but not limited to human immunodeficiency virus-1 (HIV-1), human immunodeficiency virus-2 (HIV-2), human T-cell lymphotrophic virus-I (HTLV-1), human T-cell lymphotrophic virus-II (HTLV-II), avian leukosis virus, Rous sarcoma virus, avian myeloblastosis virus, feline leukemia virus, and bovine leukemia virus. These methods may also be used with paramyxoviruses (e.g. measles virus and respiratory syncytial virus) and herpesviruses (e.g. Epstein Barr virus).

The invention also provides methods of inhibiting formation of syncytia between cells. A carbohydrate blocking agent which prevents the interaction of the virus with the cell is provided. Examples of such cells include but are not limited to T cells, B cells, and monocytes. The carbohydrate blocking agent is administered to the cells under conditions selected to allow the agent to interact with the cells, thus inhibiting formation of syncytia. The carbohydrate blocking agent in the present invention is a very water soluble cyclic oligosaccharide derivative. These methods are suitable for use with retroviruses, including but not limited to human immunodeficiency virus-1 (HIV-1), human immunodeficiency virus-2 (HIV-2), human T cell lymphotrophic virus-I (HTLV-1), human T-cell lymphotrophic virus-II (HTLV-II), avian leukosis virus, Rous sarcoma virus, avian myeloblastosis virus, feline leukemia virus, and bovine leukemia virus. These methods may also be used with paramyxoviruses (e.g. measles virus and respiratory syncytial virus) and herpesviruses (e.g. Epstein Barr Virus).

The effectiveness of the carbohydrate blocking agents on virus infection, cell to cell transmission of the virus, and syncytia formation may be assayed using procedures known in the art. A description of such procedures is given in Section 6.1, _infra_.

The invention further provides compositions of very water soluble cyclic oligosaccharide derivatives described in Section 5.1, _supra_ in combination with antiviral agents described in Section 5.2, _supra_. Such compositions may be used to inhibit the binding of a virus to a cell. In another embodiment, the compositions may be used to inhibit cell to cell transmission of the virus. In a further embodiment, such compositions may be used to inhibit syncytia formation. These compositions may be used with retroviruses, such as human immunodeficiency virus-1, human immunodeficiency virus-2, human T-cell lymphotrophic virus-I (HTLV-1), human T-cell lymphotrophic virus-II (HTLV-II), avian leukosis virus, Rous sarcoma virus, avian myeloblastosis virus, feline leukemia virus, and bovine leukemia virus. Additionally, these compositions may be used with paramyxoviruses (e.g. measles viruses or respiratory syncytial virus) or herpesviruses (e.g. Epstein Barr Virus). The effectiveness of the compositions of the present invention on virus infection or syncytia formation, may be assayed using procedures known in the art. A description of such procedures is given in Section 6.1., _infra_.

The invention further provides compositions of very water soluble cyclic oligosaccharide derivatives described in Section 5.1, _supra_. Such compositions may be used to inhibit the binding of a virus to a cell in combination with steroid or non-steroid organic compounds that contain growth modulating activity described in Section 5.3, _supra_. These compositions may be used with retroviruses, such as human immunodeficiency virus-1, human immunodeficiency virus-2, human T-cell lymphotrophic virus-I (HTLV-1), human T-cell

lymphotrophic virus-II (HTLV-II), avian leukosis virus, Rous sarcoma virus, avian myeloblastosis virus, feline leukemia virus, and bovine leukemia virus. Additionally, these compositions may be used to treat Kaposi's Sarcoma which is often manifested in AIDS patients. The effectiveness of the compositions of the present invention on virus infection or syncytia formation, may be assayed using procedures known in the art. A description of such procedures is given in Section 6.1, infra.

The invention further provides compositions of very water soluble cyclic oligosaccharide derivatives described in Section 5.1, supra. Such compositions may be used to inhibit the binding of a virus to a cell in combination with steroid or non-steroid organic compounds that contain cell growth modulating activity described in Section 5.3 and antiviral agents described in Section 5.2, supra. These compositions may be used with retroviruses, such as human immunodeficiency virus-1, human immunodeficiency virus-2, human T-cell lymphotrophic virus-I (HTLV-1), human T-cell lymphotrophic virus-II (HTLV-II), avian leukosis virus, Rous sarcoma virus, avian myeloblastosis virus, feline leukemia virus, and bovine leukemia virus. Additionally, these compositions may be used to treat Kaposi's Sarcoma which is often manifested in AIDS patients. The effectiveness of the compositions of the present invention on virus infection or syncytia formation, may be assayed using procedures known in the art. A description of such procedures is given in Section 6.1, infra.

6. EXAMPLE: SYNTHETIC CYCLODEXTRINS INHIBIT HIV INFECTION IN VITRO

The following examples are offered by way of illustration and not by way of limitation. The invention has been described herein with reference to certain preferred embodiments and examples. However obvious variations will be

apparent to those skilled in the art. The invention is not to be considered limited to the particularly exemplified embodiments.

6.1. MATERIALS AND METHODS

The fusion inhibition (syncytia) assay was performed according to published procedures (Dalgleish et al., Nature 312:763, (1984) and Sodroski et al., Nature 322: 470, (1986)). Sup-T1 cells are favored target cells for their rapid degree of cell fusion when co-cultured with HIV-1 producing cell lines. Cell culture is performed according to the method of Dalgleish et al. (Nature 312:763, (1984)). Sup-T1 cells are plated in 96 well plates (10^5 cells/well in RPMI 1640 + 10% FCS) and incubated with or without dilutions of beta-cyclodextrin tetradecasulfate (β-CD-TDS or β-CD-14S) at 37°C. HTLV-III B (HIB-1) or WMJ infected H9 cells are then added $5x10^4$/well and the number of multinucleated giant cells per 16 X field counted with a Zeiss inverted field phase contrast microscope after 18 hours. Syncytia are easily identified and inhibition of syncytia by different dilutions of β-CD-TDS can be compared with to β-CD-TDS free preparations. It will be apparent to those skilled in the art, that the formation of syncytia between cells susceptible of infection by HIV-1 and cells expressing HIV-1 envelope glycoproteins represents the same molecular phenomena involved in infection by HIV-1, but is far less hazardous and may be employed to study the mechanisms of infection and the effects of modulating factors on HIB-1 intracellular infection mechanisms, without fear of exposure to the virus itself.

For screening of different dilutions, 10-fold dilutions of a 150 micromolar solution of β-CD-TDS were prepared and directly added to syncytia assays as described above.

6.1.1. SYNCYTIA ASSAYS

For the assay of HIV-1 fusion, Supt T1 cells were used as target cells. They fuse rapidly when cocultured with HIV-1 (H9/IIIb) producing cell lines. For the assay of HIV-2 mediated cell fusion, H9 cells were used as target cells. They fuse rapidly when cocultured with HIV-2 (CEMROD-2) producing cell lines. HIV infected cells were plated in 96 well plates (10^4 cells/well in RPMI 1640 + 10% FCS) and incubated with or without dilution of compounds for 30 minutes at 37°C. Target cells are then added 5 x 10^5/well and the number of syncytia are qualitatively determined after the incubation periods described in the text.

6.1.2. REVERSE TRANSCRIPTASE ASSAY

Virus infected cells were pelleted by centrifugation and 50 μl of culture suprenatant was collected from each well for assay. Culture supernatant (25 μl) was plated in 96 well round bottomed plates and 50 μl of transcriptase cocktail was added to each well. Cocktail is 50 mM Tris-HCl, pH 8.0, 20 mM DTT, 0.6 mM $MnCl_2$, 60 mM NaCl, 0.05% NP-40, 5 μg/ml dt (oligodeoxythymidilic acid), 10 μg/ml poly A (polyriboadenylic acid), 10 μM dTTP, 1 Ci/mm ^{32}P dTTP. Plates were then incubated for 60 minutes at 37°C. After incubation samples were spotted onto Whatman DEAE-81 paper, washed and analyzed by counting or direct autoradiography.

6.1.3. VIRAL RNA ANALYSIS

Virus treated cells were grown in tissue culture and harvested for assay at 72 hrs. post-infection. The cell concentrations were then adjusted to 1 x 10^6 cells/ml with phosphate buffered saline and spotted directly onto prewetted gene screen utilizing a dot blot apparatus. The filter was then fixed in 3% NaCl, 10 mM NaH_2PO_4, (pH 7.2) containing 1% gluteraldehyde at 4°C for 1 hr. The fixed blot was then rinsed three times with proteolytic buffer (50mM EDTA, 0.1 M

Tris-HCl (pH 8.0), 20 μg/ml proteinase K) for 30 minutes at 37°C. The filter was air dried until being probed. A Sal1-Xho1 probe derived from the pIIIenv1 plasmid (a generous gift of Dr. J. Sodrofsky) and encoding the envelope region of the HXB2, HIV-1 virus was labeled by the random primer method using standard technology and hybridized to the filter as previously described (Weiner et al., 1986, Cell Immunol. 100:197).

6.1.4. STRUCTURAL STUDIES

Virus infected cells were washed and resuspended in fresh media in the presence or absence of test compounds. After a 48 hour culture period the cultures were pelleted and fixed in 1% glutaraldehyde, 0.1 M Cacodylate-HCl, pH 7.3 followed by OsO_4, and embedded in spur resin for examination of these sections by electron microscopy.

6.1.5. CYTOFLUORIMETRY

Human T cell lines were centrifuged and washed 2X in FACS buffer (1% bovine serum albumin in phosphate buffered saline with 0.1% NaN_3). The cells were resuspended at 10^7/ml and preincubated with or without β-CD-TDS as indicated. Primary antibody was then added for an additional 30 minutes, and the samples prepared as previously described (Williams et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:6488).

6.2. RESULTS

6.2.1. INHIBITION OF HIV INFECTIVITY BY SUBSTITUTED β-CYCLODEXTRINS

A prominent feature of the human lentiviruses is their ability to fuse with target cells. When analyzed in vitro on human target cell lines, HIV-mediated infection results in the formation of multinucleated giant cells or syncytia. Such syncytia have been observed to contribute to the pathology of immune cell depletion characteristic of the

infection observed in AIDS patients. This fusion process differs from the mechanism of cell entry of many other retroviruses in that fusion does not require the acidic pH of endocytotic vesicles. In vitro HIV-mediated cell fusion is thought to occur in an analogous way to virus entry in vivo.

In a series of preliminary experiments, a syncytia assay induced by HIV-1 fusion of Sup-T1 cells was performed as described in Section 6.1.1, supra. Results are illustrated in Table 1.

As shown in Table 1, at a range of concentrations of β-CD-TDS between 1.5 micromolar and 1500 micromolar, inhibition of syncytia was observed. In contrast, no inhibition of syncytia formation was observed at concentrations of 0.15 micromolar.

TABLE 1

Syncytia Inhibiting Activity:
Degree of Syncytia Formation[a]

Viral Strain	Concentration of β-CD-TDS (micromolar) 1500	150	15.0	1.50	0.15
HTLV III -B (HIV-1 IIIB)	0	2M	4L	4L	4L
HTLV III WMJ	0	0	1S	3M	4L

a Degree of syncytia formation
4=Full
0=None

Size of syncytia formed
S=Small
M=Medium
L=Large

In a series of additional experiments, analysis of HIV-1 induced syncytia was performed by coculturing H9 cells infected with the IIIb isolate with uninfected Supt-1 (a human CD4+ T cell line) cells as targets. For the assay of HIV-2, HUT 78 cells infected with the ROD-2 isolate were cocultured with H9 (a human CD4+ T cell line) target cells. β-cyclodextrin with no substituents (β-CD), with 4 sulfate groups (β-CD-4S), and with 4 propoxy groups (β-CD-4Pr) were as ineffective as media alone in inhibiting syncytia formation of HIV-1. In contrast, β-cyclodextrin bearing 14 sulfate groups per molecule (β-CD-TDS) mediated extremely high antisyncytia activity as shown in Table 2 and Figure 1.

TABLE 2

Ability of Cyclodextrins to Block HIV-1 Entry[a]

Compounds[b]	Solubility 0°C gm/100ml H_2O	Concentration (mM) β-Cyclodextrins 2.5	1.3	.63	.32	.16	.08	.04	.02	.01
Control		4L	4L	4L	4L	4L	4L	4L	4L	4L
Beta-CD	0.7	4L	4L	4L	4L	4L	4L	4L	4L	4L
Beta-CD-Pr	20	1m	4L	4L	4L	4L	4L	4L	4L	4L
Beta-CD-4-7S	13	3m	4L	4L	4L	4L	4L	4L	4L	4L
Beta-CD-14S	42	0	0	0	0	1s	2m	3L	4L	4L
Beta-CD-M	32	0	0	0	0	0	0	3m	4L	4L

a Results are expressed as the number of syncytia formed: Syncytia are scored numerically from 0 to 4, with 4 being multiple syncytia widely distributed and 0 indicating no syncytia observed. The size of the syncytia is recorded as s=small, m=medium and L=large syncytia.

b Beta-CD is unsubstituted cyclodextrin ring, Beta-CD-Pr is basic ring structure substituted with propoxy groups, Beta-CD-14S is basic ring structure completely substituted with sulfates, Beta-CD-M is basic ring structure completely substituted with methoxy groups.

β-cyclodextrin with 14 methoxy group (β-CD-14Me) exhibited a greater degree of syncytia inhibition than was observed with B-CD-TDS. The inhibition of syncytia formation was observed at doses below 80 μM.

Similar results for syncytia induction mediated by the ROD-2 isolate of HIV-2 were obtained as shown in Table 3.

TABLE 3

Ability of Cyclodextrins to Block HIV-2 Entry[a]

		Concentration of (mM) of β-Cyclodextrin								
Compounds[b]	Solubility 0° gm/100ml H_2O	2.5	1.3	.63	.32	.16	.08	.04	.02	.01
Control		4L	4L	4L	4L	4L	4L	4L	4L	4L
Beta-CD	0.7	4L	4L	4L	4L	4L	4L	4L	4L	4L
Beta-CD-14S	42	0	0	0	1s	2m	3L	4L	4L	4L
Beta-CD-M	32	0	0	0	0	0	0	3m	4L	4L

a Results are expressed as the number of syncytia formed: Syncytia were scored numerically from 0 to 4, with 4 being multiple syncytia widely distributed and 0 indicating no syncytia observed. The size of the syncytia is recorded as s=small, m=medium and L=large syncytia.

b Beta-CD is unsubstituted cyclodextrin ring, Beta-CD-14S is basic ring structure completely substituted with sulfates, Beta-CD-M is basic ring structure completely substituted with methoxy groups.

Both polysulfated β-CD and poly-methoxylayted β-cyclodextrin inhibited syncytia formation mediated by ROD-2. However, the degree of inhibition was substantially greater with β-CD-14Me.

The ability of cyclodextrins to inhibit HIV infection was assessed in vitro utilizing reverse transcriptase assays as described Section 6.1.2, supra. HTLV-IIIb cell free virus stocks were inoculated onto target cells in the presence or absence of the synthetic compounds. After a 10 day incubation period, wells were harvested and reverse transcriptase activity scored (Figure 1). β-Cyclodextrin itself (lane B) exhibited no inhibition of viral infectivity as reverse transcriptase activity appeared similar to the positive infectivity control (lane A). Partially sulfated (lane C) and propoxylated β-cyclodextrin (lane D) were in essence ineffective, with minimal inhibition of reverse transcriptase activity. In contrast, the highly sulfated β-CD-14S was extremely effective in inhibiting in vitro infectivity (lane E). While some activity was apparent at concentrations as high as 320 μM (dilution 4), reverse transcriptase activity was dramatically inhibited at concentrations as low as 80 μM (dilution 6). The β-CD-14Me (lane F) was at least as effective, with no viral reverse transcriptase activity apparent in cultures from cells in contact with virus in the presence of doses as low as 80 μM to 40 μM (dilution 7). While these results are similar to those from syncytia assays, they suggest that viral infectivity in the presence of low amounts of some substituted cyclodextrins is markedly decreased even at concentrations which do not fully inhibit syncytia formation.

6.2.2. SITES OF ACTION OF CYCLODEXTRINS

In order to determine the specific sites of viral pathogenesis interrupted by the cyclodextrin compounds, we assayed the ability of the cyclodextrins treatment to effect

reverse transcriptase levels of cells already infected with HIV-1. In contrast to the results observed when virus was added to uninfected cells, (see supra, Section 6.2.1.) no significant inhibition of reverse transcriptase was observed. This suggests that the cyclodextrins must exert their effects at earlier stages of the virus life cycle.

To determine if the cyclodextrins were interfering with steps relevant to viral replication, the ability of these compounds to inhibit viral nucleic acid synthesis was determined. Virus particles were incubated with target cells in the presence or absence of 1 mM of β-CD-14S. At 72 hours post-infection, the cells were washed and dot blot assays were performed to determine the presence or absence of viral RNA production. From target cells infected in the presence of sulfated β-cyclodextrin (Figure 3A, lane A) little or no HIV-1 specific RNA was detected. In both the unsubstituted ring and the positive control lane, substantial viral RNA was detected at this same time point. However, the inhibition of viral nucleic acid replication was not a direct effect on the replicative machinery of the virus. Infected cells incubated with sulfated β-cyclodextrin were not inhibited in their ability to produce viral RNA (Figure 3B). These results complement syncytial and reverse transcriptase assays suggesting that the antiviral effects mediated by these compounds operate early in the viral attack on the cell.

While substituted β-cyclodextrins do not inhibit viral RNA or reverse transcriptase activity in infected cells, it is possible that these compounds interfere with viral maturation. To further address this point, we analyzed virus particle production in the presence of specifically substituted cyclodextrins by electron microscopic examination. Virus infected cells were washed and resuspended in fresh media in the presence or absence of test compounds. After a 48 hour culture period, the cultures were

pelleted and fixed in 1% glutaraldehyde, 0.1 M Cacodylate-HCl, pH 7.3 followed by OsO_4 and embedded in spur resin for examination of thin sections.

Virus preparations treated in the presence of 300 μM sulfated cyclodextrins demonstrated membrane dense regions corresponding to virus budding and shedding. The HIV morphology observed in this shed virus was typical of the lentivirus family (Figure 4). Detailed morphological examination of viral particles produced in the presence of substituted cyclodextrins exhibited normal mature HIV phenotype. Additionally, we observed no significant deviation in the ratio of mature virus present after cyclodextrin treatment or in the total number of virus particles produced in the presence of these compounds. Together, these results suggest that substituted β-cyclodextrins exert no direct effects on HIV virus production or maturation. They apparently exert their effects at the cell membrane during the initial phases of attack on the cell.

6.2.3. INFLUENCE OF CYCLODEXTRINS ON INTERACTIONS WITH CD4

Both HIV-1 and HIV-2 infected cells express the CD4 human T cell surface antigen. The strict tropism observed is a manifestation of high affinity binding sites displayed on the retrovirus envelope glycoprotein which mediates binding to CD4 bearing cells. The ability of some fully substituted cyclodextrin molecules to specifically interfere with syncytia formation, reverse transcriptase activity, and the transfer of infectious viral RNA is similar to the effects of antibodies directed at the CD4 molecule. A subset of anti-CD4 antibodies specifically disrupt the viral envelopes' ability to interact with this specific receptor. A possible mode of action of these compounds is interaction with these specific sites on CD4. We have therefore determined the

ability of 1 mM substituted β-cyclodextrin to interfere with the binding of monoclonal antibodies directed at different epitopes of the CD4 glycoprotein. Flow cytometry was utilized for this investigation.

The Leu3a monoclonal antibody binds to the first cysteine bounded region of the CD4 glycoprotein and has been demonstrated to directly interfere with HIV-1, HIV-2 as well as SIV binding. The MT1151 monoclonal antibody has been mapped to the second cysteine bounded region of CD4 and is implicated in secondary structural constraints necessary for gp120-CD4 interactions. The OKt4 monoclonal antibody binds to a region distal to these first two monoclonal antibodies and is mapped to a region that is not necessary for the binding reaction between HIV virus and the CD4 positive cell.

A comparison of the reactivity patterns of these antibodies on treated and control cells demonstrates that within the limits of this analysis, none of the epitopes recognized by these monoclonal antibodies was decreased in its ability to interact with CD4 (Figure 5). These results suggest that the protein interactions important for binding to CD4, in an analgous way to virus binding, are not altered by the cyclodextrin treatment. Furthermore, the results also suggest that these compounds do not interfere with the expression of CD4 on the target cells. While these results do not rule out an effect on gp120-CD4 interactions, they indicate cyclodextrins have no direct effect on the specific protein-protein interactions at the CD4 site.

6.2.4. COMPARISON OF SUBSTITUTED CYCLODEXTRINS WITH DEXTRAN POLYSULFATES

Recent studies demonstrated the ability of the polyanionic dextran sulfates to inhibit HIV-1 infection *in vitro* (Mitsuya et al., 1988, Science 240:646-649 and Baba et al., 1988, Proc. Natl. Acad. sci. U.S.A. 85:6132-6136. The inhibition of virus activity observed with substituted β-

cyclodextrins appears similar to those reported for the dextran sulfates. We have assessed and compared the effects of these compounds and substituted β-cyclodextrins on HIV-induced syncytia formation (Figure 6A).

At 24 hours, fully sulfated β-cyclodextrins and fully methylated β-cyclodextrin exhibited similar ability to block HIV-1 infection as dextran sulfate 5000. All these compounds were more effective than the larger dextran sulfate 500,000 at blocking syncytia formation. However, there was a dramatic difference in the length of the anti-viral effect evidenced by the two classes of complex sugars (Figure 6A & B). By 48 hours, the activity of dextran sulfate 5000 had decreased by almost 90%. In the cultures treated with the methylated or sulfated cyclodextrins, the anti-syncytial activity remained unchanged during the culture period. This result became more pronounced by 144 hours in culture at which point there is little or no antiviral effect of the dextran sulfate 5000 even when present in the several hundred micromolar range. In contrast, the substituted β-cyclodextrins lost only 50% of their potency following 6 days in continuous culture.

These results have been confirmed utilizing reverse transcriptase assay as specific indicator of viral entry and infectivity (Figure 6B). Infectious virus particles were incubated in the presence of sulfated β-cyclodextrin (lane 1), dextran sulfate (lane 2) or media (lane 3) for 6 days at which time cells were resuspended in fresh media and allowed to incubate a further 10 days before being harvested and reverse transcriptase activity assayed. The sulfated β-cyclodextrin mediated significant anti-viral activity even after prolonged periods of tissue culture. This result may be related to susceptibility differences between dextran sulfate and the synthetic β-cyclodextrin to enzymatic degradation during tissue culture.

6.3 DISCUSSION

We have demonstrated dramatic antiviral effects of carbohydrate viral blocking agents, particularly fully substituted sulfated or methylated β-cyclodextrins. These antiviral effects are intimately associated with the sidechains presented by the cyclodextrin ring, and are not a function of the ring itself. Furthermore, we have examined the effect of several differently substituted molecules and have concluded that only a specific subgroup of sidechains are able to mediate significant anti-HIV effects. These compounds appear to interfere with an early event associated with HIV pathogenesis. There are several lines of evidence for these conclusions. The fully sulfated β-cyclodextrins exhibit no detectable effects on cells that have already been infected with HIV-1. We have observed no effects on reverse transcriptase activity, virus particle production, or virus particle morphology from infected cell lines incubated with concentrations of the cyclodextrins shown to be protective in syncytia assays i.e., prior to virus infection of cells. In contrast, cyclodextrin, at equivalent concentrations, blocks HIV infection of uninfected cells as demonstrated by reverse transcriptase assays and the presence of viral RNA, in addition to syncytia formation. Together, these data support a model of interference with viral function early in the encounter between virus and cell.

We have tested the ability of these compounds to specifically inhibit monoclonal antibody binding to the known receptor for HIV. The results demonstrate that there was no significant inhibition of antibodies that specifically bind to regions of CD4 necessary for viral function. This finding is important as it underscores the fact that treatment of human cells with these compounds is not likely to effect the function of this immunologically important receptor.

Several studies have indicated a general affinity for absorption (adhesion) to cell surfaces by highly hydrophilic

glycosaminoglycans and sulfated saccharides, including cyclodextrin polysulfates (Folkman et al., 1989, Science 243:1490-1493 and Folkman and Weisz, 1989, Amer. Chem. Soc. Symp. Ser. 392: 19-32) in a variety of circumstances (Folkman et al., 1989, Science 243:1490-1493; Folkman and Weisz, 1989, Amer. Chem. Soc. Symp. Ser. 392:19-32; Mitsuya et al., 1988, Science 240:646-649; Ito et al., 1987, Antiviral Res. 7:361-367; DeClerq, 1986, J. Med. Chem. 29:1561-1569; Cole et al., 1986, Nature 323:723; Hook et al., 1984, Ann. Rev. Biochem. 53:847-869; and DeSomer et al., 1968, J. Virol 2:886-893). In view of this, our results suggest that strong attachment of the agents to the cell membrane and/or the viral envelope resulting in reduced diffusion rates of receptors, thereby critically effecting the kinetics of intermolecular interactions between specific virus ligand and cell receptor molecules, by virtue of classical physical chemical principles (Lauffenburger and DeLisi, 1983, Internat. Rev. Cytol. 84:268-302).

We have compared dextran polysulfates and substituted β-cyclodextrin molecules in HIV-1 syncytia and reverse transcriptase assays. We have observed similar effects from both classes of compounds, but find a unique difference in the longevity of the antiviral effects mediated by cyclodextrins as opposed to dextran sulfates. The results indicate that the substituted cyclized compound may be more stable to prolonged contact with cells and suggests a potential for these compounds to be effective with less frequent administration without diminishing the desired antiviral effects. Finally, the observation that this class of compound interferes with early events of lentivirus pathogenesis suggests that these compounds should be useful in the treatment of other human and animal pathogens with similar mechanisms of entry.

WHAT IS CLAIMED IS:

1. A method of inhibiting infection of cells by a virus comprising

providing a carbohydrate blocking agent capable of interacting with said cells or virus to interfere with binding of the virus with the cells, said carbohydrate blocking agent comprising a cyclic oligosaccharide derivative having up to about twelve sugar monomers, wherein said derivative is characterized by a solubility at 0°C in distilled water of at least about 20 gm/100 ml; and

contacting said cells with the carbohydrate blocking agent in an amount, for a time and under conditions selected to effect interaction of the carbohydrate blocking agent with the cells to result in said interference with binding of the virus with the cells.

2. The method of claim 1, wherein said carbohydrate blocking agent is administered to said cells at a concentration ranging from approximately 0.15 micromolar to 1.5 millimolar.

3. The method of claim 1, wherein said carbohydrate agent is administered to said cells at a concentration ranging from approximately 0.15 to 150 millimolar.

4. The method of claim 1, wherein said cyclic oligosaccharide derivative contains about six to twelve sugar monomers.

5. The method of claim 4, wherein said cyclic oligosaccharide derivative contains about six to eight sugar monomers.

6. The method of claim 5, wherein said cyclic oligosaccharide is alpha-, beta-, or gamma-cyclodextrin.

7. The method of claim 1, wherein said cyclic oligosaccharide derivative is an anionic salt derivative of said oligosaccharide having substituents selected from the group consisting of sulfate, phosphate, carboxylate, and mixtures thereof associated with a physiologically acceptable cation.

8. The method of claim 1, wherein said the cyclic oligosaccharide derivative is a cyclic oligosaccharide sulfate.

9. The method of claim 8, wherein said cyclic oligosaccharide sulfate contains about 10-16 sulfate groups.

10. The method of claim 8, wherein said cyclic oligosaccharide sulfate is beta-cyclodextrin tetradecasulfate.

11. The method of claim 1, wherein said cyclic oligosaccharide derivative is a nonionic derivative of said oligosaccharide having substituents selected from the group consisting of alkyl, ester, ether, thioester, thioether, carboxylic acid, and carbohydrate moieties.

12. The method of claim 1, wherein said cyclic oligosaccharide derivative is an alkoxy substituted cyclic oligosaccharide.

13. The method of claim 12, wherein said alkoxy substituted cyclic oligosaccharide contains about 10-16 methoxy groups.

14. The method of claim 12, wherein said alkoxy substituted cyclic oligosaccharide is tetradeca methoxy beta-cyclodextrin.

15. The method of claim 12, wherein said alkoxy substituted oligosaccharide contains about 10-16 propoxy groups.

16. The method of claim 12, wherein said alkoxy substituted oligosaccharide is tetradeca propoxy beta-cyclodextrin.

17. The method of claim 1, wherein said cyclic oligosaccharide derivative contains ionic and nonionic substituents.

18. The method of claim 1, wherein said virus is retrovirus.

19. The method of claim 18, wherein said retrovirus is human immunodeficiency virus-1.

20. The method of claim 18, wherein said retrovirus is human immunodeficiency virus-2.

21. The method of claim 1, wherein said virus is a paramyxovirus.

22. The method of claim 1, wherein said virus is a herpesvirus.

23. A method of inhibiting cell to cell transmission of a virus comprising

providing a carbohydrate blocking agent capable of interacting with said cells or virus to interfere with binding of the virus with the cells, said carbohydrate blocking agent comprising a cyclic oligosaccharide derivative having up to about twelve sugar monomers, wherein said derivative is characterized by a solubility at 0°C in distilled water of at least about 20 gm/100 ml; and

contacting said cells with the carbohydrate blocking agent in an amount, for a time and under conditions selected to effect interaction of the carbohydrate blocking agent with the cells to result in said interference with cell to cell transmission of the virus.

24. The method of claim 23, wherein said carbohydrate blocking agent is administered to said cells at a concentration ranging from approximately 0.15 micromolar to 1.5 millimolar.

25. The method of claim 23, wherein said carbohydrate agent is administered to said cells at a concentration ranging from approximately 0.15 to 150 millimolar.

26. The method of claim 23, wherein said cyclic oligosaccharide derivative contains about six to twelve sugar monomers.

27. The method of claim 26, wherein said cyclic oligosaccharide derivative contains about six to eight sugar monomers.

28. The method of claim 27, wherein said cyclic oligosaccharide is alpha-, beta-, or gamma-cyclodextrin.

29. The method of claim 23, wherein said cyclic oligosaccharide derivative is an anionic salt derivative of said oligosaccharide having substituents selected from the group consisting of sulfate, phosphate, carboxylate, and mixtures thereof associated with a physiologically acceptable cation.

30. The method of claim 23, wherein said derivative of cyclic oligosaccharide derivative is cyclic oligosaccharide sulfate.

31. The method of claim 30, wherein said cyclic oligosaccharide sulfate contains about 10-16 sulfate groups.

32. The method of claim 30, wherein said cyclic oligosaccharide sulfate is beta-cyclodextrin tetradecasulfate.

33. The method of claim 23 wherein said cyclic oligosaccharide derivative is a nonionic derivative of said oligosaccharide having substituents selected from the group consisting of alkyl, ester, ether, thioester, thioether, carboxylic acid, and carbohydrate moieties.

34. The method of claim 23, wherein said cyclic oligosaccharide derivative is an alkoxy substituted cyclic oligosaccharide.

35. The method of claim 34, wherein said alkoxy substituted cyclic oligosaccharide contains about 10-16 methoxy groups.

36. The method of claim 34, wherein the alkoxy substituted cyclic oligosaccharide is tetradeca methoxy beta-cyclodextrin.

37. The method of claim 34, wherein said alkoxy substituted cyclic oligosaccharide contains about 10-16 propoxy groups.

38. The method of claim 34, wherein said alkoxy substituted cyclic oligosaccharide is tetradeca propoxy beta-cyclodextrin.

39. The method of claim 23, wherein said cyclic oligosaccharide derivative contains ionic and nonionic substituents.

40. The method of claim 23, wherein said virus is retrovirus.

41. The method of claim 40, wherein said retrovirus is human immunodeficiency virus-1.

42. The method of claim 40, wherein said retrovirus is human immunodeficiency virus-2.

43. The method of claim 23, wherein said virus is a paramyxovirus.

44. The method of claim 23, wherein said virus is a herpesvirus.

45. A method of inhibiting formation of syncytia between cells infected with a virus comprising

providing a carbohydrate blocking agent capable of interacting with said cells or virus, said carbohydrate blocking agent comprising a cyclic oligosaccharide derivative having up to about twelve sugar monomers, wherein said derivative is characterized by a solubility at 0°C in distilled water of at least about 20 gm/100 ml; and

administering said agent to said cells under conditions selected to allow effect interaction of said cyclic oligosaccharide derivative with said cells thereby inhibiting formation of syncytia.

46. The method of claim 45, wherein said agent is are administered to said cells at concentrations ranging from approximately 0.15 micromolar to 1.5 millimolar.

47. The method of claim 45, wherein said agent is are administered to said cells at concentrations ranging from approximately 0.15 to 150 millimolar.

48. The method of claim 45, wherein said cyclic oligosaccharide derivative contains about six to twelve sugar monomers.

49. The method of claim 48, wherein said cyclic oligosaccharide derivative is a cyclic molecule having six to eight sugar monomers.

50. The method of claim 49, wherein said cyclic oligosaccharide is alpha-, beta-, or gamma-cyclodextrin.

51. The method of claim 45, wherein said cyclic oligosaccharide derivative is an anionic salt derivative of said oligosaccharide having substituents selected from the group consisting of sulfate, phosphate, carboxylate, and mixtures thereof associated with a physiologically acceptable cation.

52. The method of claim 45, wherein said cyclic oligosaccharide derivative is cyclic oligosaccharide sulfate.

53. The method of claim 55, wherein said cyclic oligosaccharide derivative contains about 10-16 sulfate groups.

54. The method of claim 52, wherein said cyclic oligosaccharide sulfate is beta-cyclodextrin tetradecasulfate.

55. The method of claim 52, wherein said cyclic oligosaccharide derivative is a nonionic derivative of said oligosaccharide having substituents selected from the group consisting of alkyl, ester, ether, thioester, thioether, carboxylic acid, and carbohydrate moieties.

56. The method of claim 55, wherein said cyclic oligosaccharide derivative is an alkoxy substituted cyclic oligosaccharide.

57. The method of claim 56, wherein said alkoxy substituted cyclic oligosaccharide contains about 10-16 methoxy groups.

58. The method of claim 56, wherein the alkoxy substituted cyclic oligosaccharide is tetradeca methoxy beta-cyclodextrin.

59. The method of claim 56, wherein said alkoxy substituted oligosaccharide contains about 10-16 propoxy groups.

60. The method of claim 56, wherein said alkoxy substituted oligosaccharide is tetradeca propoxy beta-cyclodextrin.

61. The method of claim 45, wherein said cyclic oligosaccharide derivative contains ionic and nonionic substituents.

62. The method of claim 45, wherein said virus is retrovirus.

63. The method of claim 62, wherein said retrovirus is human immunodeficiency virus-1.

64. The method of claim 62, wherein said retrovirus is human immunodeficiency virus-2.

65. The method of claim 45, wherein said virus is a paramyxovirus.

66. The method of claim 45, wherein said virus is a herpesvirus.

67. A composition for inhibiting undesired infection of cells by a virus in mammals, including humans, comprising (1) a derivative of a cyclic oligosaccharide in combination with (2) an antiviral agent, in which the derivative is characterized by a solubility at 0°C in distilled water at least about 20 gm/100 ml of water.

68. The method of claim 67, wherein said cyclic oligosaccharide derivative contains about six to twelve sugar monomers.

69. The method of claim 68, wherein said cyclic oligosaccharide derivative contains about six to eight sugar monomers.

70. The composition of claim 69, wherein said cyclic oligosaccharide is alpha-, beta-, or gamma-cyclodextrin.

71. The composition of claim 67, wherein the cyclic oligosaccharide derivative is an anionic salt derivative of said cyclodextrin having substituents selected from the group consisting of sulfate, phosphate, carboxylate, nitrate and mixtures thereof associated with a physiologically acceptable cation.

72. The composition of claim 67, wherein said derivative of said cyclic oligosaccharide is a cyclodextrin sulfate.

73. The composition of claim 72, wherein said cyclic oligosaccharide sulfate contains about 10-16 sulfate groups.

74. The composition according to claim 71, wherein said cyclic oligosaccharide sulfate is beta-cyclodextrin tetradeca sulfate.

75. The composition of claim 67, wherein said cyclic oligosaccharide derivative is a nonionic derivative of said oligosaccharide having substituents selected from the group consisting of alkyl, ester, ether, thioester, thioether, carboxylic acid, and carbohydrate moieties.

76. The composition of claim 67, wherein said cyclic oligosaccharide derivative is an alkoxy substituted cyclic oligosaccharide.

77. The method of claim 76, wherein said alkoxy substituted cyclic oligosaccharide contains about 10-16 methoxy groups.

78. The composition of claim 76, wherein said alkoxy substituted cyclic oligosaccharide is tetradeca methoxy beta-cyclodextrin.

79. The composition of claim 67, wherein said cyclic oligosaccharide derivative contains ionic and nonionic substituents.

80. The composition of claim 67, wherein said antiviral agents is a nucleoside derivative.

81. The composition of claim 67, wherein said antiviral agent is a CD4 peptide or analog thereof.

82. The composition of claim 67, wherein said antiviral agent is hypericin.

83. The composition of claim 67, wherein said virus is a retrovirus.

84. The composition of claim 67, wherein said retrovirus is human immunodeficiency virus-1.

85. The composition of claim 84 wherein said retrovirus is human immunodeficiency virus-2.

86. The composition of claim 67, wherein said virus is a paramyxovirus.

87. The composition of claim 67, wherein said virus is a herpesvirus.

88. A composition for inhibiting cell to cell transmission of the virus in mammals, including humans comprising (1) a derivative of a cyclic oligosaccharide in

combination with (2) an antiviral agent, in which the derivative is characterized by a solubility at 0°C in distilled water at least about 20 gm/100 ml of water.

89. The composition of claim 88, wherein said cyclic oligosaccharide derivative contains about six to twelve sugar monomers.

90. The composition of claim 88, wherein said cyclic oligosaccharide derivative contains about six to eight sugar monomers.

91. The composition of claim 88, wherein said cyclic oligosaccharide is alpha-, beta-, or gamma-cyclodextrin.

92. The composition of claim 88, wherein said cyclic oligosaccharide derivative is an anionic salt derivative of said oligosaccharide having substituents selected from the group consisting of sulfate, phosphate, carboxylate, and mixtures thereof associated with a physiologically acceptable cation.

93. The composition of claim 88, wherein said derivative of cyclic oligosaccharide derivative is cyclic oligosaccharide sulfate.

94. The composition of claim 93, wherein said cyclic oligosaccharide sulfate contains about 10-16 sulfate groups.

95. The composition of claim 93, wherein said cyclic oligosaccharide sulfate is beta-cyclodextrin tetradecasulfate.

96. The composition of claim 88, wherein said cyclic oligosaccharide derivative is a nonionic derivative of said oligosaccharide having substituents selected from the group consisting of alkyl, ester, ether, thioester, thioether, carboxylic acid, carbohydrate, halide, and alkyl halide moieties.

97. The composition of claim 88, wherein said cyclic oligosaccharide derivative is an alkoxy substituted cyclic oligosaccharide.

98. The composition of claim 97, wherein said alkoxy substituted cyclic oligosaccharide contains about 10-16 methoxy groups.

5 99. The composition of claim 973, wherein the alkoxy substituted cyclic oligosaccharide is tetradeca methoxy beta-cyclodextrin.

100. The composition of claim 98, wherein said alkoxy substituted cyclic oligosaccharide contains about 10-16 propoxy groups.

101. The composition of claim 98, wherein said alkoxy substituted cyclic oligosaccharide is tetradeca propoxy beta-cyclodextrin.

102. The composition of claim 88, wherein said cyclic oligosaccharide derivative contains ionic and nonionic substituents.

103. The composition of claim 88, wherein said virus is retrovirus.

104. The composition of claim 103, wherein said retrovirus is human immunodeficiency virus-1.

105. The composition 6of claim 103, wherein said retrovirus is human immunodeficiency virus-2.

106. The composition of claim 88, wherein said virus is a paramyxovirus.

107. The composition of claim 88, wherein said virus is a herpesvirus.

108. The composition of claim 105, wherein said antiviral agents is a nucleoside derivative.

109. The composition of claim 105, wherein said antiviral agent is a CD4 peptide or analog thereof.

110. The composition of claim 105 wherein the antiviral agent is hypericin.

111. A composition for inhibiting syncytia formation between virus-infected cells in mammals, including humans, comprising (1) a derivative of a cyclic oligosaccharide cyclodextrin in combination with (2) an antiviral agent wherein said derivative is characterized by a solubility at 0°C in distilled water at least about 20 gm/100 ml of water.

112. The composition of claim 111, wherein said cyclic oligosaccharide derivative contains about six to twelve sugar monomers.

113. The composition of claim 112, wherein said cyclic oligosaccharide derivative contains about six to eight sugar monomers.

114. The composition according to claim 113, wherein said derivative of alpha-, beta-, or gamma-cyclodextrin is a cyclodextrin sulfate.

115. The composition according to claim 111, the derivative of said oligosaccharide is an anionic salt derivative of said cyclodextrin having substituents selected from the group consisting of sulfate, phosphate, carboxylate, nitrate and mixtures thereof associated with a physiologically acceptable cation.

116. The composition according to claim 115, wherein said cylcodextrin sulfate contains about 10-16 sulfate groups.

117. The composition according to claim 115, wherein the cyclodextrin sulfate is beta-cyclodextrin tetradeca sulfate.

118. The composition according to claim 111, wherein said derivative of alpha-, beta-, or gamma-cyclodextrin is an alkoxy cyclodextrin.

119. The composition of claim 118, wherein said alkoxy cyclodextrin contains about 10-16 methoxy groups.

120. The composition of claim 118, wherein said alkoxy cyclodextrin is tetradeca methoxy beta-cyclodextrin.

121. The composition of claim 118, wherein said alkoxy cyclodextrin contains about 10-16 propoxy groups.

122. The composition of claim 118, wherein said alkoxy cyclodextrin is tetradeca propoxy beta-cyclodextrin.

123. The composition of claim 111, wherein said antiviral agents is a nucleoside derivative.

124. The composition of claim 111, wherein said antiviral agent is a CD4 peptide or analog thereof.

125. The composition of claim 111, wherein said antiviral agent is hypericin.

126. The composition of claim 111, wherein said virus is a retrovirus.

127. The composition of claim 126, wherein said retrovirus is human immunodeficiency virus-1.

128. The composition of claim 126, wherein said retrovirus is human immunodeficiency virus-2.

129. The composition of claim 111, wherein said virus is a paramyxovirus.

130. The composition of claim 111, wherein said virus is a herpesvirus.

131. A composition for inhibiting undesired infection of cells by a retrovirus in mammals including humans, comprising (1) cyclic oligosaccharide derivative in combination with (2) a latent cell growth modulating steroid or non-steroid organic compound wherein said steroid does not possess glucocorticoid activity, and wherein cyclic oligosaccharide derivative is characterized by a solubility at 0°C in distilled water at least about 20 gm/100 ml of water.

132. The composition of claim 131, wherein said cyclic oligosaccharide derivative contains about six to twelve sugar monomers.

133. The composition of claim 131, wherein said cyclic oligosaccharide derivative contains about six to eight sugar monomers.

134. The composition of claim 131, wherein said cyclic oligosaccharide is alpha-, beta-, or gamma-cyclodextrin.

135. The composition of claim 131 wherein the derivative of cyclic oligosaccharide derivative is an anionic salt derivative of said oligosaccharide having substituents selected from the group consisting of sulfate, phosphate, carboxylate, and mixtures thereof associated with a physiologically acceptable cation.

136. The composition of claim 131, wherein said derivative of cyclic oligosaccharide derivative is cyclic oligosaccharide sulfate.

137. The composition of claim 136, wherein said cyclic oligosaccharide sulfate contains about 10-16 sulfate groups.

138. The composition of claim 136, wherein said cyclic oligosaccharide sulfate is beta-cyclodextrin tetradecasulfate.

139. The composition of claim 131 wherein said cyclic oligosaccharide derivative is a nonionic derivative of said oligosaccharide having substituents selected from the group consisting of alkyl, ester, ether, thioester, thioether, carboxylic acid, and carbohydrate moieties.

140. The composition of claim 131, wherein said cyclic oligosaccharide derivative is an alkoxy substituted cyclic oligosaccharide.

141. The composition of claim 140, wherein said alkoxy substituted cyclic oligosaccharide contains about 10-16 methoxy groups.

142. The composition of claim 140, wherein the alkoxy substituted cyclic oligosaccharide is tetradeca methoxy beta-cyclodextrin.

143. The composition of claim 140, wherein said alkoxy substituted cyclic oligosaccharide contains about 10-16 propoxy groups.

144. The composition of claim 140, wherein said alkoxy substituted cyclic oligosaccharide is tetradeca propoxy beta-cyclodextrin.

145. The composition of claim 140, wherein said cyclic oligosaccharide derivative contains ionic and nonionic substituents.

146. The composition of claim 131, wherein said retrovirus is human immunodeficiency virus-1.

147. The composition of claim 131, wherein said retrovirus is human immunodeficiency virus-2.

148. The composition of claim 131 wherein the steroid in cortisone, hydrocortisone, cortexolone, or tetrahydrocorticol.

149. The composition according to claim 131 wherein the non-steroid compound is interferon-alpha, interferon-beta or interferon-interferon.

150. The composition according to claim 131 wherein the non-steroid compound is interleukin-1 to interleukin-2.

151. A composition for inhibiting undesired infection of cells by a retrovirus in mammals including humans, comprising (1) cyclic oligosaccharide derivative in combination with (2) a latent cell growth modulating steroid or non-steroid organic compound and (3) an antiviral agent, wherein said steroid does not possess glucocorticoid activity, and wherein cyclic oligosaccharide derivative is characterized by a solubility at 0°C in distilled water at least about 20 gm/100 ml of water.

152. The composition of claim 151, wherein said cyclic oligosaccharide derivative contains about six to twelve sugar monomers.

153. The composition of claim 151, wherein said cyclic oligosaccharide derivative contains about six to eight sugar monomers.

154. The composition of claim 151, wherein said cyclic oligosaccharide is alpha-, beta-, or gamma-cyclodextrin.

155. The composition of claim 151 wherein the derivative of cyclic oligosaccharide derivative is an anionic salt derivative of said oligosaccharide having substituents selected from the group consisting of sulfate, phosphate, carboxylate, and mixtures thereof associated with a physiologically acceptable cation.

156. The composition of claim 151, wherein said derivative of cyclic oligosaccharide derivative is cyclic oligosaccharide sulfate.

157. The composition of claim 156, wherein said cyclic oligosaccharide sulfate contains about 10-16 sulfate groups.

158. The composition of claim 156, wherein said cyclic oligosaccharide sulfate is beta-cyclodextrin tetradecasulfate.

159. The composition of claim 151 wherein said cyclic oligosaccharide derivative is a nonionic derivative of said oligosaccharide having substituents selected from the group consisting of alkyl, ester, ether, thioester, thioether, carboxylic acid, and carbohydrate moieties.

160. The composition of claim 151, wherein said cyclic oligosaccharide derivative is an alkoxy substituted cyclic oligosaccharide.

161. The composition of claim 160, wherein said alkoxy substituted cyclic oligosaccharide contains about 10-16 methoxy groups.

162. The composition of claim 160, wherein the alkoxy substituted cyclic oligosaccharide is tetradeca methoxy beta-cyclodextrin.

163. The composition of claim 160, wherein said alkoxy substituted cyclic oligosaccharide contains about 10-16 propoxy groups.

164. The composition of claim 160, wherein said alkoxy substituted cyclic oligosaccharide is tetradeca propoxy beta-cyclodextrin.

165. The composition of claim 160, wherein said cyclic oligosaccharide derivative contains ionic and nonionic substituents.

166. The composition of claim 151, wherein said retrovirus is human immunodeficiency virus-1.

167. The composition of claim 151, wherein said retrovirus is human immunodeficiency virus-2.

168. The composition of claim 151 wherein the steroid in cortisone, hydrocortisone, cortexolone, or tetrahydrocorticol.

169. The composition according to claim 151 wherein the non-steroid compound is interferon-alpha, interferon-beta or interferon-interferon.

170. The composition according to claim 151 wherein the non-steroid compound is interleukin-1 to interleukin-2.

171. The composition according to claim 151 wherein said antiviral agent is nucleoside derivative.

172. The composition according to claim 151 wherein said antiviral agent is a CD4 peptide or analog thereof.

173. The composition of claim 151 wherein said antiviral agent is hypericin.

FIG. 1A

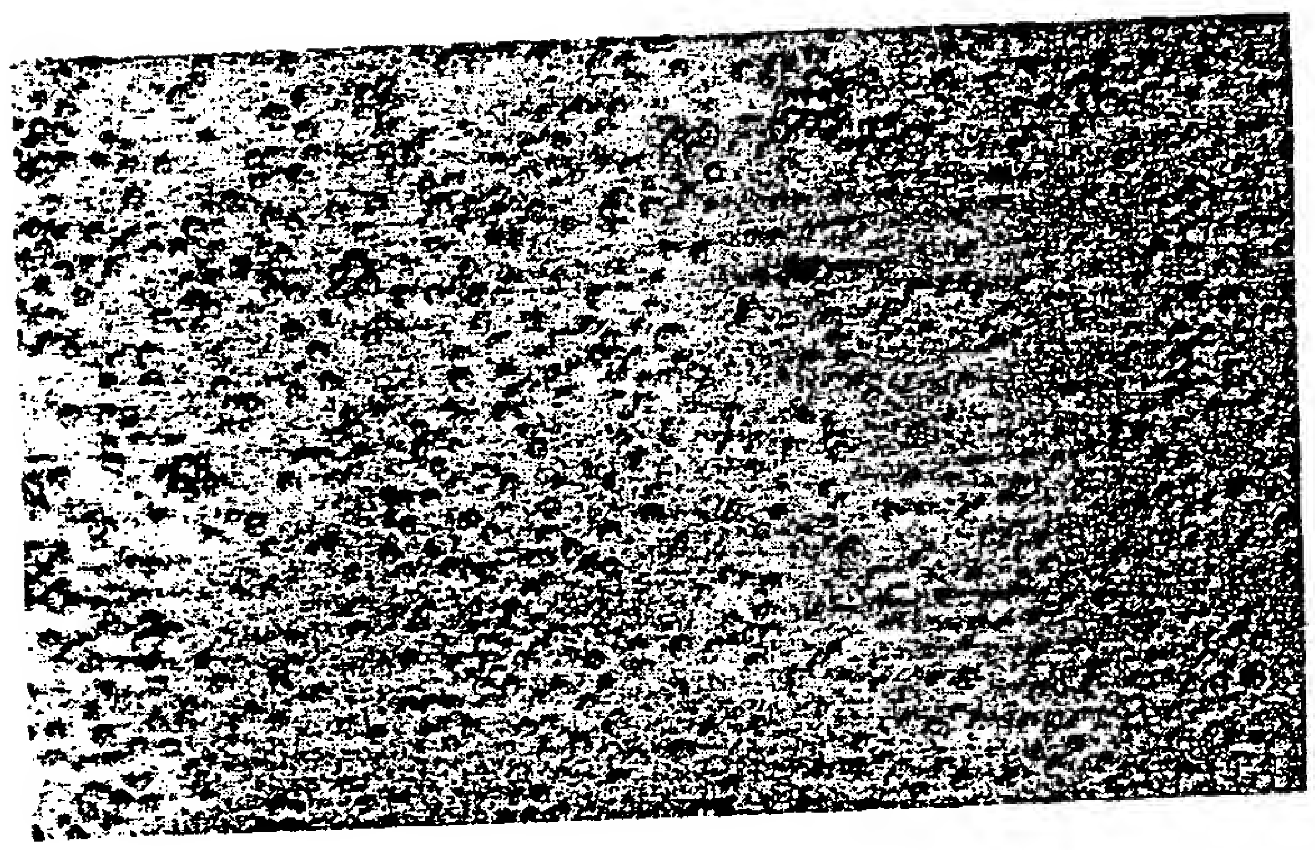


FIG. 1B

FIG. 2

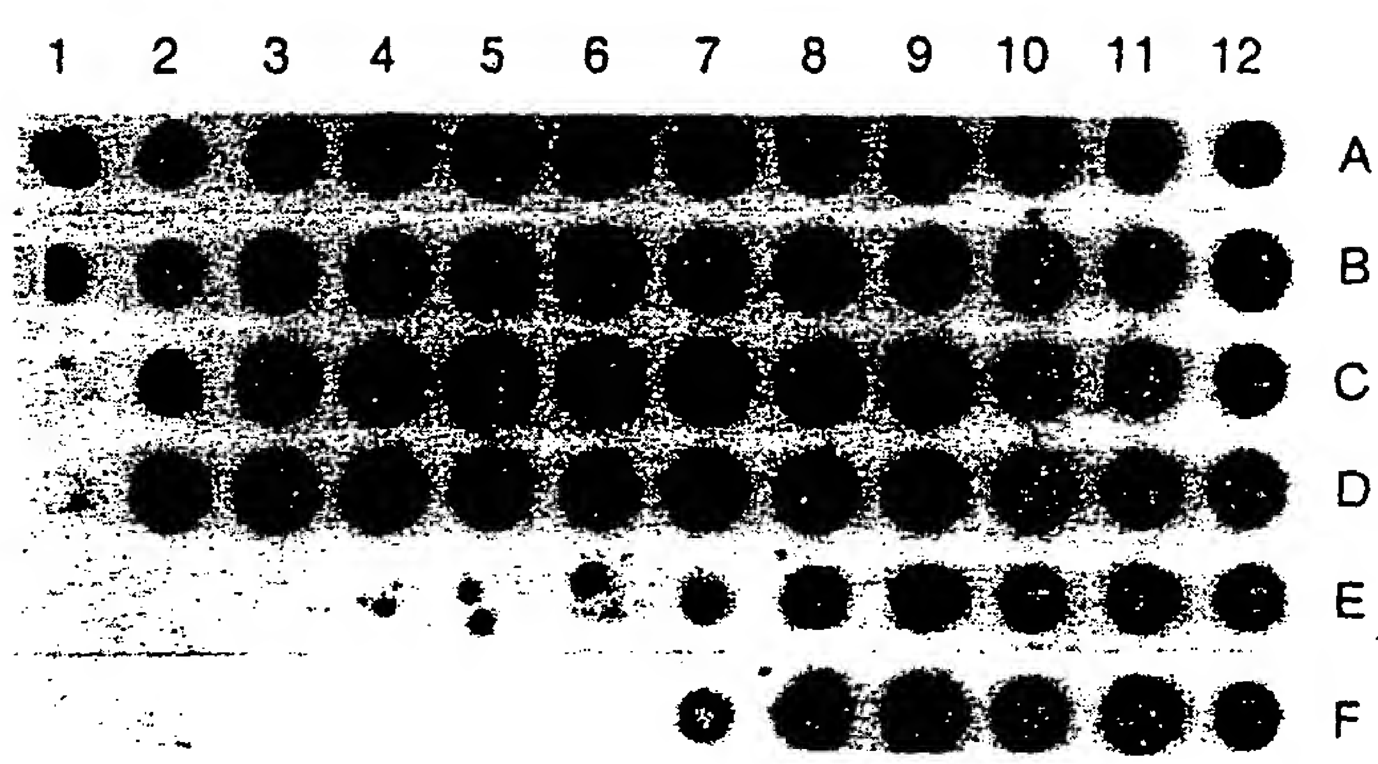
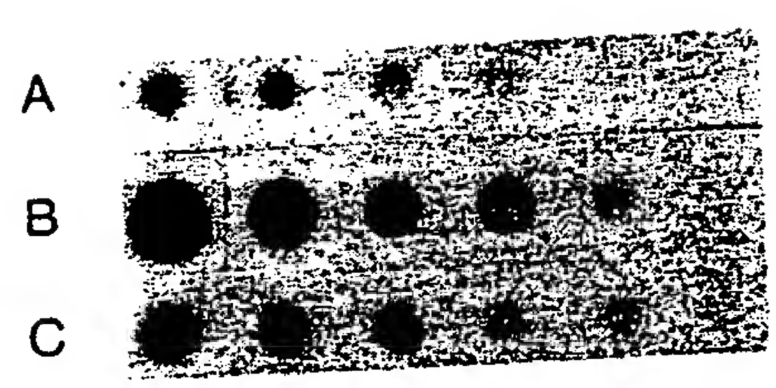


FIG. 3A



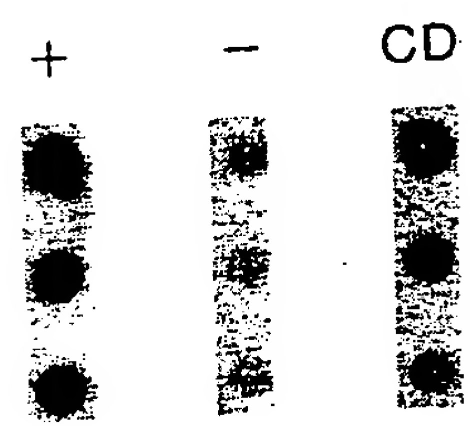
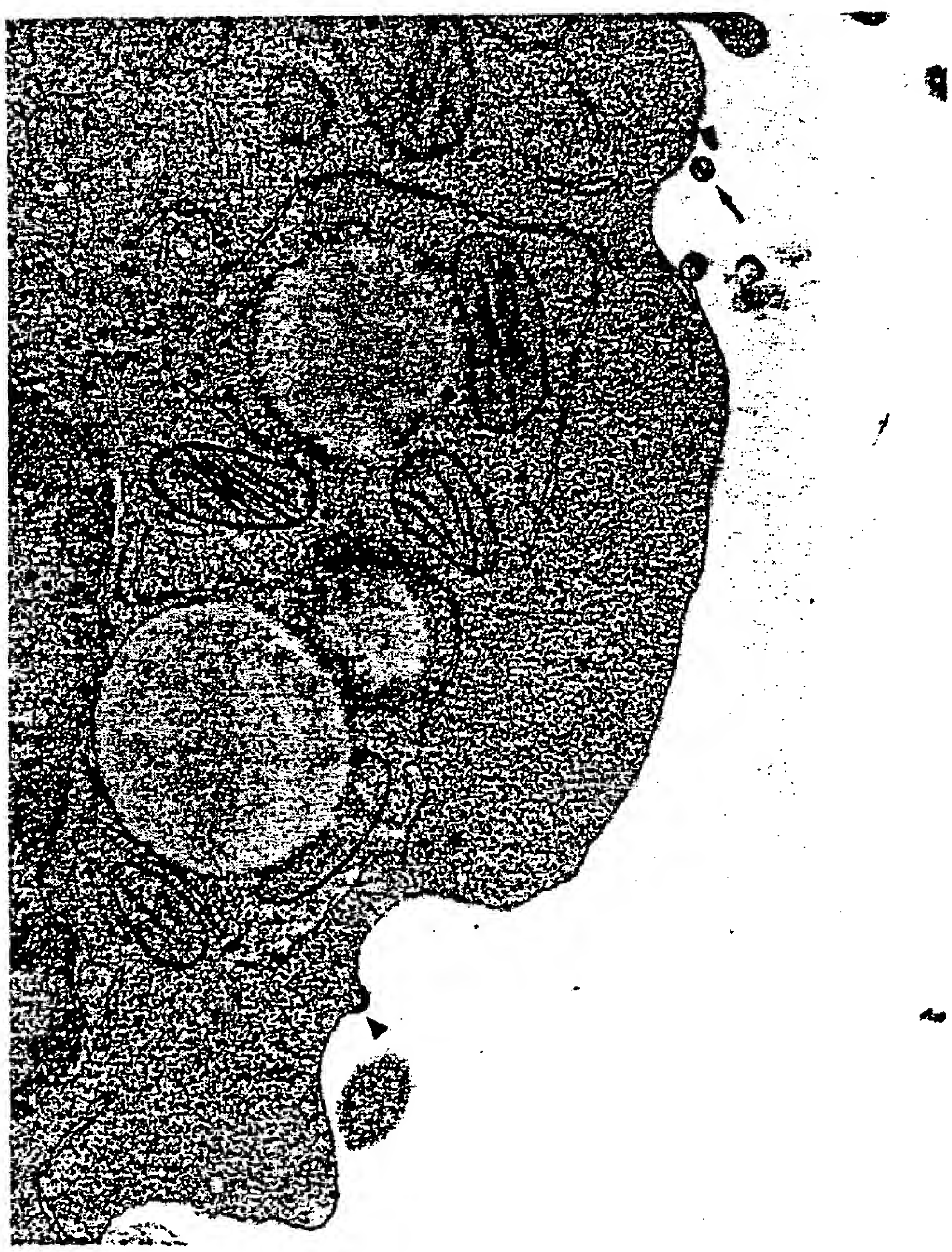


FIG. 3B

FIG. 4



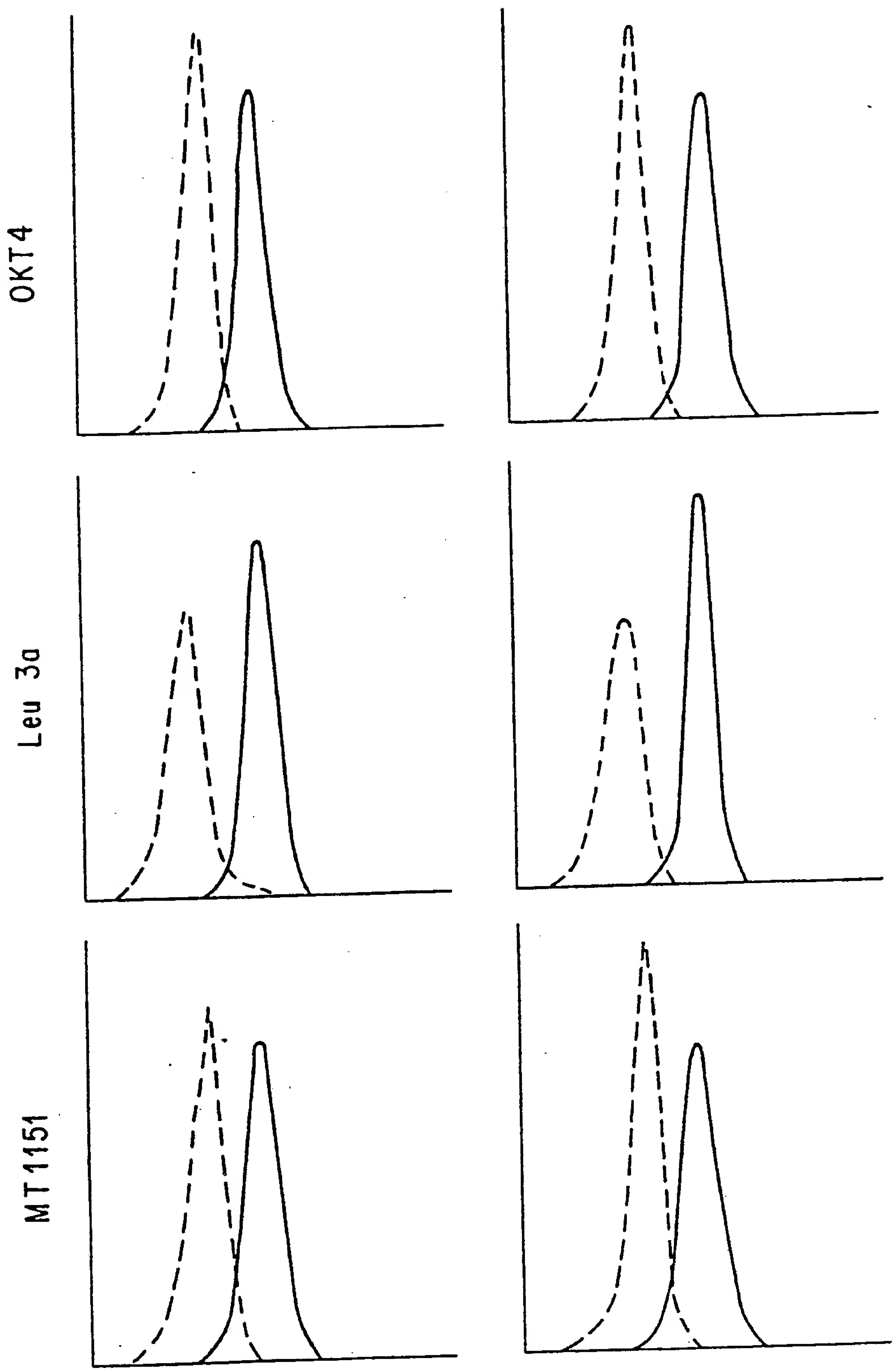


FIG. 5

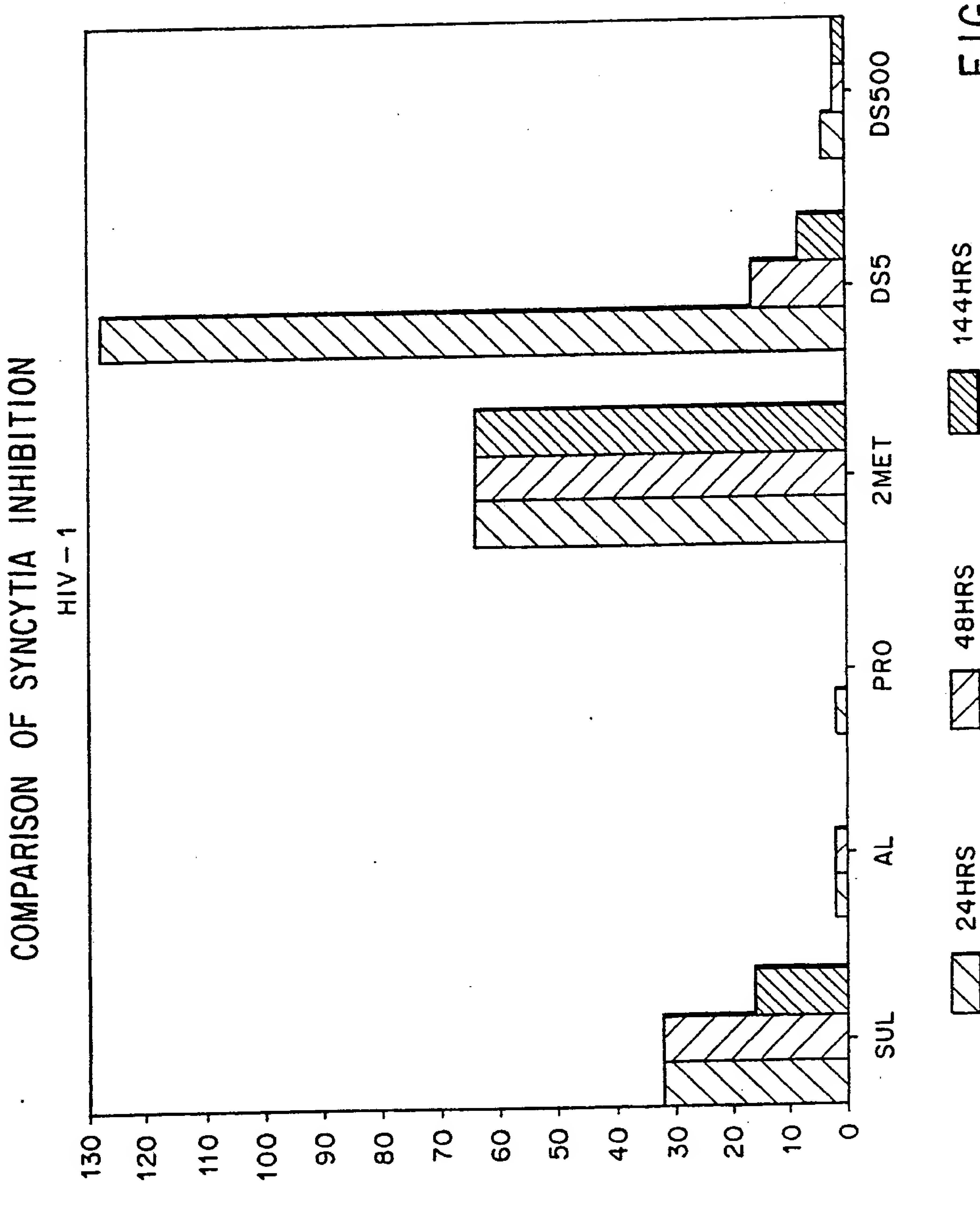
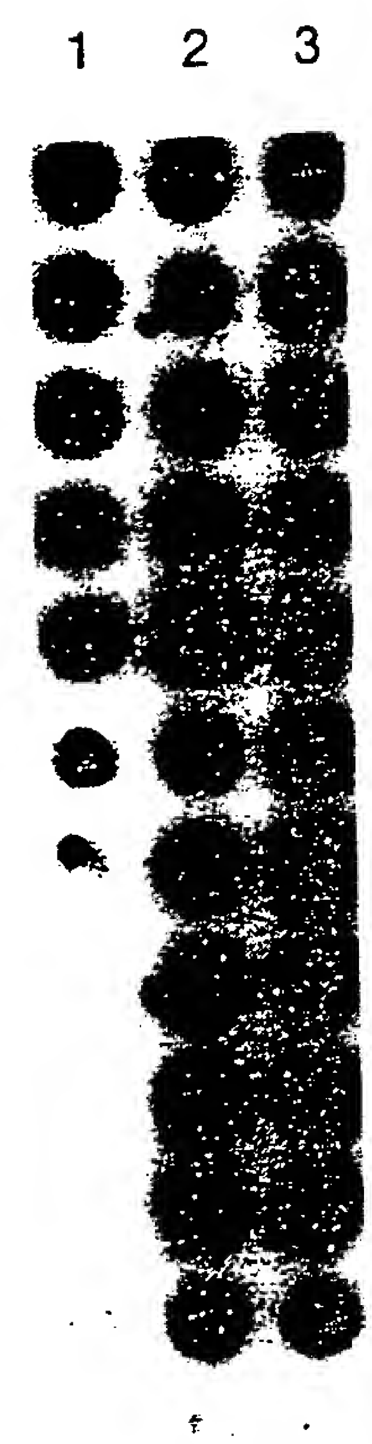

COMPARISON OF SYNCYTIA INHIBITION
HIV-1
130
120
110
100
90
80
70
60
50
40
30
20
10
0
SUL
AL
PRO
2MET
DS5
DS500
24HRS
48HRS
144HRS

FIG. 6a

FIG.6b



INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/02944

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): C12N 5/00; A61K 31/735

U.S.Cl.: 435/240.2; 514/54,58

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/240.2; 514/54,58,885

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Databases: Chemical Abstracts (CAS) 1967-1989; Biological Abstracts (Biosis) 1969-1989; Dialog File Biosci; 1964-1989 See Attachment for Search terms

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y,P	US,A 4,783446 (Neushul) 8 November 1988 See entire document.	1-11,17-33, 39-55,61-66, 67-75,79-96, 102-117, 123-139, 145-159, 165-173
Y	Antimicrobial Agents and Chemotherapy, Volume 31, Issued October 1987, Nakashima et al., "Purification and characterization of an avian immuno-deficiency virus reverse transcriptase inhibitor, sulfated polysacharides extracted from sea algae, pages 1524-1528, see entire document.	1-11,17-33, 39-55,61-75, 79-96,102-117, 123-139, 145-159, 165-173

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16 October 1989	07 NOV 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Gail F. Knox

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Science, Volume 240, Issued 29 April 1988, Mitsuya et al., "Dextran sulfate suppression of viruses in the HIV family: Inhibition of Virion binding to $CD4^+$ Cells, pages 646-649, See entire document.	1-11,17-33, 39-55,61-75, 79-96, 102-117, 123-139, 145-159 165-173.

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ...., because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers..........., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers__________, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Attachment to Form PCT/IPEA/408

Part I. SEARCH TERMS

cyclodextrins
cycloamylose
AIDS
HIV
Polysaccharides
Oligosaccharides
Antiviral
Interferon
Interleukin
Cortexolone
desoxycortisol